In Vitro Effects of Inhalational Anesthetics on Viscosity of Human Blood

Marjam G. Behar, Ph.D., and S. Craighead Alexander, M.D.†*

Human blood was equilibrated with halothane (1.2 per cent), cyclopropane (20 per cent), nitrous oxide (70 per cent), and diethyl ether (4 per cent and 15 per cent). The remaining gas in each instance was 5 per cent carbon dioxide in oxygen (carbogen). Blood viscosity was measured before and after equilibration with anesthetic, or anesthetic-free carbogen, utilizing a specially adapted micro-cone plate viscometer. Clinical concentrations of the inhalational anesthetics produced no changes in whole blood viscosity. However, high concentrations of diethyl ether in the range of 250 mg./100 ml., produced a small but statistically significant increase in viscosity.

Flow through blood vessels is determined by the perfusion pressure, cross-sectional area of the vessels, and blood viscosity. Only the first two factors have been commonly considered in studies of cardiac output and organ blood flow, while the viscosity of blood usually has been assumed to remain constant. However, the flow properties of blood can be changed by numerous factors, including burns and other tissue injury,[1-5] extensive operation,[9] cardiopulmonary bypass,[10,11] and several kinds of shock.[7,12-14] Furthermore, certain drugs, notably dextrans, may affect blood viscosity markedly.[10,14-17] The effects of anesthetic agents on blood viscosity are largely unexplored. In the present study, *in vitro* effects of inhalational anesthetics on human whole blood viscosity are reported, and some basic rheological concepts are discussed.

Methods

Blood samples were obtained from 33 normal adult volunteers. Twenty milliliters of forearm venous blood was sampled without stasis, through a 19-gauge needle into a 20 ml. glass syringe containing 0.15 ml. of heparin sodium solution (1,000 units/ml.). The final heparin concentration was 7.5 units/ml. blood.[18]

Each sample was divided into three parts. Viscosity of a 6 ml. portion was measured without further handling (unequilibrated control). Viscosity of a second 7 ml. fraction was measured after tonometry with the humidified anesthetic gas mixture (experimental sample). The balance gas in each instance was 5 per cent CO_2 in O_2 (carbogen), which provided near physiologic levels of oxygen and carbon dioxide. The final third of the sample (equilibrated control) was equilibrated with humidified anesthetic-free carbogen, and its viscosity was then measured.

Tonometric Procedure. Measured concentrations of diethyl ether were delivered by means of a Vernitrol vaporizer* and calibrated flowmeters. A Fluotec† vaporizer was employed for vaporization of halothane. Delivery of cyclopropane and nitrous oxide was metered by calibrated rotameters.

A rotating tonometer was used to perform nine experiments with 15 per cent diethyl ether, three with 1.2 per cent halothane, three with 20 per cent cyclopropane, and one with 70 per cent nitrous oxide. This system consisted of two, 285 ml. glass vessels in a

* Instructor, Department of Anesthesia, University of Pennsylvania.

† Assistant Professor, Department of Anesthesia, University of Pennsylvania. Recipient of U. S. Public Health Service Career Development Award 5-K3-GM-25, 153-02.

Received from the Department of Anesthesia, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania. Accepted for publication February 21, 1966.

This work was supported (in part) by Grant GM-09070-01 from the National Institutes of Health, U. S. Public Health Service, and by a grant from the John A. Hartford Foundation.

* Ohio Chemical Company.

† Fraser-Sweatman, Inc.

TABLE 1. Blood Viscosity: Mean Control Values (33 samples)

	Shear Rate (sec.$^{-1}$)									
	230		115		46		23		11.5	
Hct. (%)	Visc. (cp.)	S.E.	Visc. (cp.)	S.E.	Visc. (cp.)	S.E.	Visc. (cp.)	S.E.	Visc. (cp.)	S.E.
47.2	4.25	0.09	4.63	0.10	5.54	0.14	6.65	0.18	7.93*	0.25

* 21 samples only.

Mean viscosity values and standard errors are shown in centipoises (cp.) for 33 samples of normal whole human blood at shear rates ranging from 230 to 11.5 sec.$^{-1}$.

37.0° C. water bath.[19] Experimental and control samples were equilibrated simultaneously for one hour with gas flows through the tonometer flasks of 100 ml./minute.

In 17 additional studies an Instrumentation Laboratory Tonometer was used for simultaneous equilibration of control and experimental blood samples in 200 ml. flasks. Six experiments were performed with 4 per cent diethyl ether, five with 70 per cent nitrous oxide, three with 20 per cent cyclopropane and three with 1.2 per cent halothane. Gas flow through the flasks was 500 ml./minute and equilibration time was 12 minutes. Analysis of blood concentrations of halothane and diethyl ether by gas chromatography † proved that equilibration was complete in all but those equilibrated with 15 per cent diethyl ether. In the latter a mean ether concentration of 245 mg./100 ml. was found.

A comparison of the effects on blood viscosity of the two tonometric systems revealed no appreciable differences.

All glassware and other pieces of equipment which came in contact with blood were siliconized before each experiment.§

Analytical Procedures. Viscosity was measured with a Brookfield micro cone-plate viscometer, Model LVT,¶ [20] specially adapted so that blood gas tensions could be maintained constant during measurement of viscosity. The sample cup was fitted with an injection port at the base, a purging port above the testing area, and a water jacket. The sample cup was purged with the appropriate humidified gas mixture for 10 minutes prior to the anaerobic injection of the 1.0 ml. blood sample and during the entire period when viscosity measurements were being made. Temperature within the cup was maintained at 37.0° ± 0.2° by circulating water from a constant temperature bath through the jacket. The instrument was calibrated with National Bureau of Standards Oils H, I, and K. Measurements of viscosity were made in duplicate at shear rates of 230, 115, 46, 23, and 11.5 seconds^{-1} (inverse seconds), after an initial shearing at 230 sec.$^{-1}$ for 5 minutes.# [18]

The reproducibility of the method for measuring viscosity was determined by analyzing 11 samples from one blood specimen at each shear rate. The 95 per cent confidence limits were found to lie within 6 per cent of the mean at all shear rates except 11.5 sec.$^{-1}$ where the 95 per cent confidence band included the mean ± 13 per cent. Other workers [21] have found good agreement between values obtained with the Brookfield device and the G.D.M. viscometer,[22] which permits measurements over a wider range of rates (from 100 to 0.02 sec.$^{-1}$) with a reputed accuracy of 2 per cent.

Hematocrits were determined in duplicates by Wintrobe technique. Experimental results were rejected if the variation in hematocrit between two simultaneous equilibrated sam-

† Method of R. A. Butler, to be published.

§ Siliclad (Clay-Adams, Inc., New York City).

¶ Brookfield Engineering Laboratories, Inc., Stoughton, Mass.

This initial shearing is necessary because at a constant shear rate blood viscosity decreases with time, reaching a stable value in about 4 minutes. This property of blood is known as thixotropy.

TABLE 2. Blood Viscosity Changes Due to Tonometry
(33 samples)

	Shear Rate (sec.$^{-1}$)									
	230		115		46		23		11.5	
Δ Hct. (%)	Δ Visc. (cp.)	S.E.	Δ Visc. (cp.)	S.E.	Δ Visc. (cp.)	S.E.	Δ Visc. (cp.)	S.E.	Δ Visc. (cp.)	S.E.
−0.01	+0.11	0.04	+0.13	0.05	+0.20	0.07	+0.23	0.08	+0.41*	0.14

* 21 samples only.

Mean blood viscosity changes due to tonometry are shown in centipoises (cp.), together with their standard errors. Values are presented for shear rates ranging from 230 to 11.5 sec.$^{-1}$

ples (control and experimental) was more than ± 0.5 per cent in a given study or if hemolysis was observed in either sample.

Statistical analysis was performed by means of Student's paired *t*-test.

Results

Mean values for viscosity and hematocrit of whole normal human blood at various shear rates are listed in table 1.

Mean changes in blood viscosity and hematocrit due to tonometry are presented in table 2. The equilibration procedure caused a small increase in blood viscosity. Although this change was statistically significant, it represented an increase in blood viscosity of less than 6 per cent at the lowest shear rate and about 3 per cent at all other shear rates. The mean decrease in hematocrit was only 0.01 per cent.

The mean changes in viscosity produced by anesthetic agents are given in table 3. The values in the table represent the differences between samples of blood equilibrated with the anesthetic-free carbogen and those equilibrated concurrently with the anesthetic gas in carbogen. None of the anesthetic agents in clinical concentrations produced statistically significant changes in blood viscosity, at any shear rate. However, higher concentrations of diethyl ether, in the range of 245 mg./100 ml., produced a small but statistically signifi-

TABLE 3. Blood Viscosity Changes Due to Anesthetic Agents

			Shear Rate (sec.$^{-1}$)									
			230		115		46		23		11.5	
Drug	Studies	Δ Hct. (%)	Δ Visc. (cp.)	S.E.	Δ Visc. (cp.)	S.E.	Δ Visc. (cp.)	S.E.	Δ Visc. (cp.)	S.E.	Δ Visc. (cp.)	S.E.
Halothane 1.2%	6	−0.1	0.00	0.08	0.00	0.10	−0.03	0.11	+0.06	0.13	+0.08†	0.30
Cyclopropane 20%	6	+0.1	+0.05	0.04	+0.05	0.06	+0.05	0.07	+0.04	0.06	+0.10‡	0.09
Nitrous Oxide 70%	6	−0.5	+0.03	0.04	+0.05	0.03	+0.04	0.04	+0.02	0.07	−0.12†	0.09
Diethyl Ether 4%	6	+0.1	+0.09	0.13	+0.10	0.16	+0.13	0.21	+0.14	0.25	+0.06†	0.30
Diethyl Ether 15%*	9	0	+0.13	0.05	+0.17	0.07	+0.34	0.12	+0.61	0.13	+0.57‡	0.23

* Incomplete equilibration—mean blood ether concentration 245 mg. per cent.

† Measured in 5 studies only.

‡ Measured in 3 studies only.

Mean blood viscosity changes due to anesthetic agents are shown in centipoises (cp.) along with their standard errors. The entries in the table represent the differences between the values for blood samples equilibrated with carbogen and those equilibrated concurrently with the anesthetic gas in carbogen, at shear rates ranging from 230 to 11.5 inverse seconds (sec.$^{-1}$).

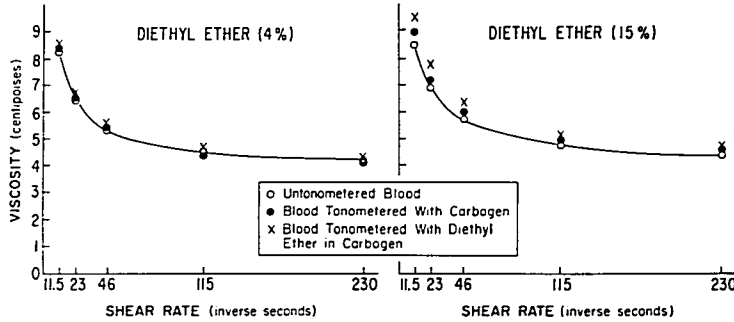

FIG. 1. Rheological behavior of normal whole human blood. The figure shows the viscosity-shear rate relationships without tonometry, following tonometry with anesthetic free carbogen, and after tonometry with diethyl ether in carbogen.

cant increase in viscosity ($P < 0.05$ at shear rates of 230 and 115 sec.$^{-1}$; $P < 0.02$ at 46 sec.$^{-1}$; and $P < 0.01$ at 23 sec.$^{-1}$).

In figure 1 is a graphical presentation of the rheological behavior of blood as measured without tonometry, following tonometry with anesthetic-free carbogen, and after tonometry with 4 per cent and 15 per cent diethyl ether.

Discussion

Interpretation of the data from this investigation depends upon a familiarity with certain basic rheological concepts. Therefore, the flow properties of fluids in general and blood in particular will be considered first. There will follow an examination of the results of this study, and finally a discussion of the relevance of these findings to the *in vivo* situation.

Basic Rheologic Concepts. When a liquid flows through a cylindrical tube, friction at the walls of the tube exerts a backward "drag" on the outermost layer of fluid; this retardation is transmitted in decreasing degree to the inner concentric layers of fluid. Consequently, a velocity gradient is present in the flowing stream. The flow velocity is maximum at the center and decreases in a parabolic manner, approaching zero at the walls. The change in velocity per unit distance across the tube diameter is termed shear rate described in units of cm. /second /cm., or simply seconds^{-1}. The force per unit area required to produce a given rate of shear is the shear stress expressed in dynes/cm.^{2}. The ratio of shear stress to shear rate defines the viscosity of a fluid. Essentially this ratio expresses the internal friction or resistance of fluid to flow. The basic unit for viscosity is the poise (poise = dyne sec./cm.^{2}), but a more convenient unit is the centipoise (cp.) = 1/100 poise.

In water and other liquids, the ratio shear stress /shear rate is constant; therefore viscosity is independent of shear rate, as illustrated in figure 2. Such fluids are termed "ideal" or "Newtonian," after Sir Isaac Newton who first postulated such a relationship. However, in many complex fluids, such as blood, the ratio of shear stress to shear rate is not constant; and viscosity varies with shear rate. Such fluids are termed "non-Newtonian." As shear rate increases the viscosity of blood is diminished (fig. 1). The decrease in viscosity with a rise in shear rate has been attributed to dissociation of red cell-protein clusters and their alignment in the flowing stream.[23] With this orientation, a smaller force (shear stress) is required to maintain fluid motion.

Although shear rate is the primary determinant of blood viscosity, other variables are also important; these include temperature,

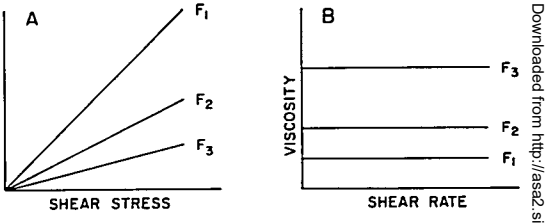

FIG. 2. Rheological behavior of newtonian fluids. The straight line relationship of shear stress to shear rate is shown in figure 2A for three different Newtonian fluids F_1, F_2 and F_3. In figure 2B is the relationship of viscosity to shear rate for the same three fluids.

hematocrit, and factors influencing erythrocyte aggregation. As temperature is reduced, blood viscosity is increased. This effect is more pronounced below 27° C. Blood viscosity increases with hematocrit, and the elevation becomes exponential as hematocrit exceeds 50 per cent.[18]

Increased clumping of erythrocytes elevates blood viscosity and thereby diminishes flow in the microvasculature. Plasma proteins are those constituents that most strongly affect red cell aggregation.[24, 25] The effect of plasma proteins on blood viscosity varies with molecular weight, configuration, and charge. Fibrinogen, a fibrillar protein with a high molecular weight and low electronegativity has been shown to be the chief factor in erythrocyte aggregation.[24, 26] With major trauma or burns, a marked increase in fibrinogen concentration occurs, viscosity is elevated, and blood flow is impaired. In contrast, albumin, which is a small, low molecular weight protein with a high degree of electronegativity, inhibits cell aggregation and therefore diminishes viscosity. The effects of globulins on cell aggregation vary with their structure and molecular weight as predicted by the principles outlined above.

Artificially produced polymers, as well as plasma proteins, likewise influence blood viscosity. Low molecular weight dextran (less than 60,000) has been found useful in preventing and reversing the increased erythrocyte aggregation which occurs in several pathological states.[10, 14-16] In contrast, high molecular weight dextran (over 150,000) promotes erythrocyte aggregation.[14, 16]

Data of This Study. Measurements of blood viscosity obtained in this study (table 1) are in close agreement with the work of others.[18, 20, 23, 27] The slight increase in viscosity caused by tonometry has not been reported previously. Most likely it is related to changes in plasma proteins caused by mechanical trauma.[11] Such phenomena were not further investigated in this work.

Our data indicate that the *in vitro* addition to blood of clinical concentrations of cyclopropane, halothane, nitrous oxide or diethyl ether produces no measurable rheological change. Yet there is a theoretical reason to expect that inhalational anesthetics could alter blood viscosity. It has been shown that inhalational anesthetics combine with blood proteins to form complexes which are stable as long as the anesthetic agent is present.[28] The fact that protein configuration influences blood viscosity raises the question as to whether the presence of anesthetic-protein complexes can alter the rheological behavior of blood. Since changes in viscosity were not found, it would appear that anesthetic molecules of low molecular weight do not appreciably alter the structure or molecular weight of proteins, which are considerably larger. However, the fact that concentrations of diethyl ether in blood of 245 mg./100 ml. produced an increase in viscosity may indicate that protein-anesthetic complexes can affect blood viscosity when the concentration is high.

Changes of Blood Viscosity in Intact Man and Animals. Although the addition to blood of clinical concentrations of anesthetic agents *in vitro* does not affect blood viscosity, the rheological effects of general anesthesia in intact man and animals are largely uninvestigated. Eckstein *et al.* reported that blood viscosity in the dog is increased during ether anesthesia and diminished during pentobarbital narcosis.[29] However, in their investigation

viscosity was determined with a capillary viscometer only at one shear rate, and change in hematocrit could not be ruled out as the cause of observed alterations. More recently, blood viscosity of man has been reported to be reduced about 3 per cent during halothane or thiopental anesthesia, and increased about 2 per cent during cyclopropane administration.[30] In that study *relative* viscosity was determined only at one shear rate, and the findings could be accounted for by the reported changes in hematocrit. Furthermore, the significance of the small changes cannot be assessed without further information on the reproducibility of the method used to determine blood viscosity.

A final problem lies in the interpretation of viscosity measurements at the shear rates used in this study. *In vivo* shear rates have been estimated to range from 108 sec.$^{-1}$ in the normal aorta to 10 sec.$^{-1}$ in arterioles of 100 μ diameter.[23] Shear rates in the capillaries and venules are much lower since flow rates even at the center of the vessel are only 0.4 mm./second, and viscosity is therefore markedly increased in these vessels. Since the instrument used in the present study is adequate for viscosity measurements only at shear rates from 230 sec.$^{-1}$ to 11.5 sec.$^{-1}$, it was not possible to make measurements at the shear rates which exist in capillaries and venules. However, we consider it unlikely that anesthetic agents which do not affect blood viscosity at the shear rates measured could change viscosity significantly at lower shear rates.

Summary

Blood viscosity increases to levels that could impair flow following trauma, extensive surgery, cardiopulmonary by-pass, burns and other tissue injury. These changes in viscosity are attributed to alterations of plasma proteins, particularly an increase in fibrinogen concentration.

Inhalational anesthetics form complexes with plasma proteins which are stable as long as the anesthetic is present. In order to determine the effects of inhalational anesthetics on blood viscosity, human blood was equilibrated in a tonometer with 1.2 per cent halothane, 20 per cent cylcopropane, 70 per cent nitrous oxide and 4 per cent and 15 per cent diethyl ether. The remaining gas in each instance was 5 per cent carbon dioxide in oxygen.

Viscosity of the blood was determined before and after equilibration with anesthetics or anesthetic-free carbogen in a specially adapted microcone plate viscometer while gas tensions were held constant. Tonometry without anesthetics was shown to produce a 3 to 6 per cent increase in viscosity. None of the inhalational anesthetics produced additional changes in blood viscosity when used in clinical concentrations. However, higher concentrations of diethyl ether, in the range of 250 mg./100 ml. blood, produced a small but statistically significant increase in viscosity. Some basic rheological concepts are discussed in relation to these data.

References

1. Bergentz, S. E., Gelin, L. E., Rudenstam, C. M., and Federfeldt, B.: The viscosity of whole blood in trauma, Acta Chir. Scand. 126: 289, 1963.
2. Bigelow, W. G., Heimbecker, R. O., and Harrison, R. C.: Intravascular agglutination (sludged blood), vascular stasis and sedimentation rate of the blood in trauma, Arch. Surg. 59: 667, 1949.
3. Brooks, F., Dragstedt, L. R., Warner, L., and Knisely, M. H.: Sludged blood following severe thermal burns, Arch. Surg. 61: 387, 1950.
4. Gelin, L. E.: Studies in anemia of injury, Chir. Scand., Suppl. 210, 1956.
5. Gelin, L. E.: The significance of intravascular aggregation of blood cells following injury, Soc. Int. Chir. 18: 4, 1959.
6. Gelin, L. E.: Disturbance of the flow properties of blood and its counteraction in surgery, Acta Chir. Scand. 122: 287, 1961.
7. Knisely, M. H., Eliot, T. S., and Block, E. H.: Sludged blood in traumatic shock, Arch. Surg. 51: 220, 1945.
8. Knisely, M. H., Block, E. H., Eliot, T. S., and Warner, L.: Sludged blood, Science 106: 431, 1947.
9. Eiken, O.: Thrombotic occlusion of experimental grafts as a function of the regional blood flow, Acta Chir. Scand. 121: 410, 1961.
10. Long, D. M., Jr., Sanchez, L., Varco, R. L., and Lillehei, C. W.: Use of low molecular dextran and serum albumin as plasma expanders in extracorporeal circulation, Surgery 50: 12, 1961.
11. Wright, E. S., Sarkozy, E., Harpur, E. R., Dobell, A. R. C., and Murphy, D. R.: Plasma protein denaturation in extracorporeal cir-

culation, J. Thorac. Cardiov. Surg. 44: 550, 1962.
12. Heimbecker, R. O., and Bigelow, W. G.: Intravascular agglutination of erythrocytes (sludged blood) and traumatic shock, Surgery 28: 461, 1950.
13. Knisely, M. H., and Block, E. H.: Microscopic observations of intravascular agglutination of red cells and consequent sludging of the blood in human diseases, Anat. Rec. 82: 426, 1942.
14. Shoemaker, W. C.: Studies on viscosity alterations in shock. I. Effect of high and low molecular dextrans upon plasma and red cell volumes, Arch. Surg. 87: 355, 1963.
15. Bergentz, S. E., Gelin, L. E., Rudenstam, C. M., and Federfeldt, B.: Indications for use of low viscous dextran in surgery, Acta Chir. Scand. 122: 343, 1961.
16. Gelin, L. E., and Shoemaker, W. C.: Hepatic blood flow and microcirculatory alteration induced by dextrans of high and low viscosity, Surgery 49: 713, 1961.
17. Schenk, W. G., Jr., Delin, N. A., Domanig, E., Hahnloser, P., and Hoyt, R. K.: Blood viscosity as a determinant of regional blood flow, Arch. Surg. 89: 783, 1964.
18. Rand, P. W., Lacombe, E., Hunt, H. E., and Austin, W. H.: Viscosity of normal human blood under normothermic and hypothermic conditions, J. Appl. Physiol. 19: 117, 1964.
19. Severinghaus, J. W., Stupfel, M., and Bradley, A. F.: Accuracy of blood pH and P_{CO_2} determinations, J. Appl. Physiol. 9: 189, 1956.
20. Wells, R. E., Jr., Denton, R., and Merril, E. W.: Measurements of viscosity of biologic fluids by cone-plate viscometer, J. Lab. Clin. Med. 57: 646, 1961.
21. Charm, S., McCornis, W., Tepda, C., and Kurland, G.: Effect of a fatty meal on whole blood viscosity, J. Appl. Physiol. 18: 1217, 1963.
22. Gilinson, P. J., Dauwalter, C. R., and Merril, E. W.: A rotational viscometer using an A.C. torque to balance loop and air bearing, Trans. Soc. Rheology 7: 319, 1963.
23. Wells, R. E., Jr., and Merril, E. W.: Influence of flow properties of blood upon viscosity-hematocrit relationships, J. Clin. Invest. 41: 1591, 1962.
24. Wells, R. E., Jr.: Rheology of blood in the microvasculature, New Eng. J. Med. 270: 832 and 889, 1964.
25. Wells, R. E., Jr., and Merril, E. W.: Shear rate dependence of viscosity of blood: Interaction of red cells and plasma proteins, Trans. Soc. Rheology 6: 19, 1962.
26. Merril, E. W., Cokelet, G. C., Britten, A., and Wells, R. E., Jr.: Non-Newtonian rheology of human blood-effect of fibrinogen deduced by substraction, Circ. Res. 13: 48, 1963.
27. Wells, R. E., Jr., and Merril, E. W.: Shear rate dependence of the viscosity of whole blood and plasma, Science 133: 763, 1961.
28. Featherstone, R. M., Muehlbaecher, C. A., DeBon, F. L., and Forsaith, J. A.: Interactions of inert anesthetic gases with proteins, ANESTHESIOLOGY 22: 977, 1961.
29. Eckstein, D. B., and Gregg, D. E.: Blood viscosity under different experimental conditions and its effect on blood flow, Amer. J. Physiol. 135: 772, 1942.
30. Albert, S. N., Jain, S. C., and Shadid, J. N.: Studies on blood viscosity and its significance in anesthesia, Med. Ann. D. C. 33: 372, 1964.

LOCAL ANESTHETICS The effect of *pH* on the action of lidocaine and dibucaine on desheathed rabbit nonmyelinated nerve fibers was studied using the size of the C elevation of the compound action potential and the electrical threshold as indices of the degree of block. The rate of block was found to be greater when the anesthetic was applied in neutral rather than in alkaline solution. When a nerve was exposed to a low concentration of dibucaine in alkaline solution, little block developed but when the nerve was transferred to an anesthetic-free solution at neutral *pH* complete block developed rapidly. Conduction was restored upon returning the nerve to the alkaline anesthetic solution. These results provide support for the hypothesis that the cation is the active form of local anesthetics and that the uncharged molecule is important only for penetration into the nerve. (*Ritchie, J. M., and others: The Active Structure of Local Anesthetics, J. Pharmacol. Exp. Ther. 150: 152 (Oct.) 1965.*)